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Original Article

Validation of a high-throughput and robust technique: BACs-on-beads assay (KaryoLite BoBs) for pre-implantation aneuploidy screening

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ABSTRACT

Objective: This study aims to validate the BACs-on-Beads (BoB) technology as a robust and high throughput method for pre-implantation genetic screening (PGS) for aneuploidy.
Material and methods: The performances with respect to the sensitivity, specificity, success rate and detection rate of this technique from new BoBs technology and traditional array chromosomal genomic hybridization (aCGH) were compared. And the use of BoBs as a screening tool for euploid embryos in PGS was evaluated.
Result: In the first part of validation study, there were total 75 embryos completed PGS by both BoBs and aCGH. The success rate of PGS was 97.4%, and the results showed 100% concordance between BoBs and aCGH for aneuploidy. In the second part, a total 219 embryos were involved. The success rate of PGS by BoBs was 100%. BoBs identified 28% (62/219) euploidy which were further confirmed to be euploidy by aCGH.
Conclusion: This new strategic approach using BoBs as a first tier PGS screening tool and aCGH as a confirmatory tool can increase the throughput of PGS with a reduced cost and time to meet the demand in high volume units.

Introduction

The development of in-vitro fertilization (IVF) gives an opportunity for infertile couples to conceive. However, the global pregnancy rate from IVF has maintained at around 35–40% in the past decade [1–3] despite the advancement and innovation in reproductive technology. Several investigators have confirmed that numerical chromosomal abnormalities were common in embryos produced from IVF [4–7], ranging from 60% abnormal embryos in women younger than 35 years to 80% in women 41 years and older [7]. The high frequency of numerical chromosomal abnormalities is likely to have a substantial effect on the IVF failure [8].

Preimplantation Genetic Screening (PGS) aims to improve the IVF outcome by identifying chromosomally normal (euploid) embryo for transfer by assessing its ploidy status. The latest systemic review of randomised trials [9] on PGS-v2 showed that PGS can improve the implantation rates and ongoing pregnancy rates. A variety of genetic testing technologies, which assess the whole chromosome complement (22 autosome and XY chromosomes), are available for PGS. Array Comparative Genomic Hybridization (aCGH) based on BAC-DNA (Bacterial Artificial Chromosomes or oligonucleotide) probes is one of the most widely used currently. However, this technology is expensive, requires molecular skilled staff and is difficult to scale up.

Recently there is a newly developed technology called KaryoLite BoBs that can detect numerical and arm-specific aneuploidies in all 24 chromosomes in a single assay. It is a bead-based multiplex

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Table 1
Results of KaryoLite BoBs and aCGH on detecting chromosomal abnormalities in validation study.

	KaryoLite BoBs	aCGH
Percentage (n) of Euploidy	28.0% (21)[a]	28.0% (21)
Percentage (n) of Aneuploidy	72.0% (54)	72.0% (54)
Total (n)	100%	100%

[a] One case of partial 9p and 12q deletion was missed by KaryoLite.

assay, based on bacterial artificial chromosomes (BACs)-on-beads and the Luminex xMAP technology [10]. In brief, it utilizes BAC-DNA probes coupled to fluorescently labelled polystyrene microsphere beads. Each bead is coupled with three different BAC DNA clones representing the p-and q-arms of all autosomes as well as sex chromosomes. For the analysis, fluorescently labelled sample and reference DNAs are individually hybridized to the beads before calculating the sample versus reference ratio for each genomic location represented by the BAC clones. Samples are analysed in a 96-well format on a Luminex® LX200 instrument and generated data are interpreted by the dedicated software BoBsoft™. In this way, the aneuploidy status of all chromosomes can be rapidly and easily assessed.

The application of BoBs was first demonstrated by Gross et al. [11] showing that the BoBs approach is a rapid and reliable test for detecting aneuploidies and microdeletions in a prenatal setting. The accuracy of KaryoLite BoBs on detecting unbalanced gains or losses of genetic material across the genome in prenatal diagnosis was previously demonstrated [10]. Previous studies using KaryoLite BoBs for analysing the product of conception also demonstrated that it was superior to the conventional cytogenetic evaluation in the areas of success rate, cost, turnaround time and subjective result interpretation [12–15].

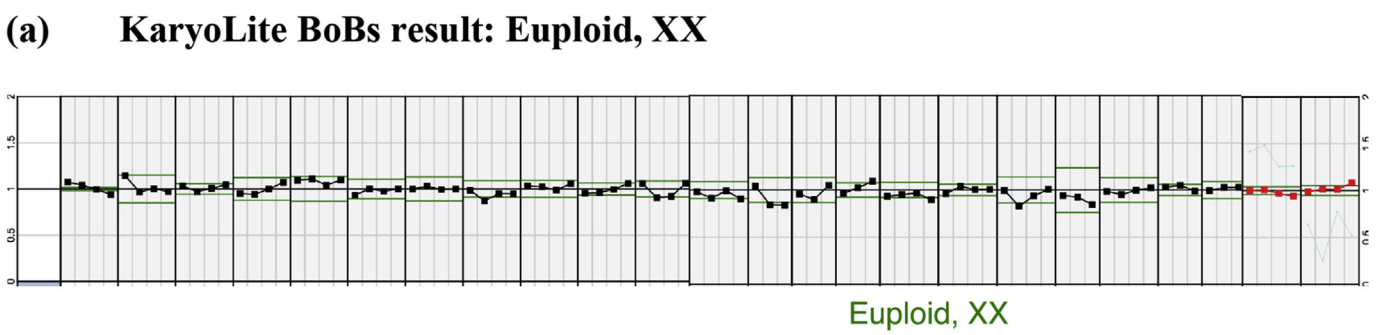


Fig. 1. (a) Typical normal euploid result by KaryoLite, (b) The discordance case missed by KaryoLite BoBs in validation study.

In the current study, we described the use of this newly established KaryoLite BoBs assay for PGS in identifying and eliminating aneuploidy, and provided comparisons with aCGH with respect to the sensitivity, specificity, success rate and detection rate of aneuploidy. In addition, we performed a retrospective analysis to evaluate the performance of PGS using this KaryoLite BoBs technology as the first line screening tool followed by aCGH for confirmation of euploid embryos. Our study identify KaryoLite BoBs as a cost-effective approach with high throughput for PGS to meet the demand in high volume units.

Materials and methods

This is a validation study conducted in Pre-implantation Genetic Diagnosis Laboratory of The Chinese University of Hong Kong in Hong Kong and Shenzhen, China, over a two-year recruitment period (January 2012 to June 2014). This study was divided into 2 parts. In Part I of study, blastomeres or trophectoderm (TE) cells were biopsied from donated embryos from three Assisted Reproductive Technology units including Hong Kong, Hainan and Suzhou, for preimplantation genetic screening (PGS) with both KaryoLite BoBs and aCGH (standard method) techniques for analysis. The performances from these two methods were compared. In Part II of study, the performance of a new approach using KaryoLite BoBs as the first line screening tool and followed by aCGH for PGS in identifying euploid embryos was evaluated. This study was approved by local institutional board (Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee, CRE-2010.432). And, the study is in compliance with the Declaration of Helsinki.

Single cell whole genome amplification

One to two blastomeres from cleavage stage embryos or an average of five TE cells from blastocysts were biopsied using laser pulses of a non-contact laser (Saturn Active, Research Instrument, UK) to breach the zona pellucida. Each cell sample was placed in a 0.2 ml PCR tube containing 2.0 ul phosphate-buffered saline (Cell Signaling Technologies, Beverly, MA, USA). Whole genome amplification was performed using the PicoPLEX WGA kit (PerkinElmer, Inc.) according to manufacturer's instructions. Amplified products were assessed by agarose gel electrophoresis to confirm the success of amplification.

Comprehensive chromosome screening

The KaryoLite™ BoBs™ assay was performed according to the manufacturer's protocol. In brief, 2 ul of single cell WGA products were amplified with biotin-labelled dNTP mix for 60–90 min. After the removal of unbound biotin labelled dNTPs, the samples were hybridized to the KaryoLite™ BoBs™ bead set at 52 °C in shaking incubator (800 rpm) for 16 h. Luminex® 200™ instrument was used for signal detection and initial data processing was performed using Luminex 100 IS Software version 2.3.182 (Luminex Corp., Austin, TX). The cvs data file generated was then imported to BoBsoft™ v2.0 (PerkinElmer, Waltham, MA) for aneuploidy detection.

PGS arrayCGH analysis

The whole genome amplified DNA samples isolated from biopsied samples subjected to KaryoLite BoBs were also processed for aCGH analysis according to the 24 sure protocol (BlueGnome/Illumina) [16] for the part I validation study. Visualization and reporting of aneuploidy were performed using BlueFuse Software (BlueGnome/Illumina) on a per chromosome basis. The scanning data were then analysed and quantified by algorithm fixed settings in BlueFuse Multi Software (BlueGnome, Cambridge, UK), a software package that automatically performed the steps of grid placement, quantification, normalization and post-processing. Only whole chromosome aneuploidies (gains and losses) were scored. For the second part, a high-resolution oligo microarray from Agilent technology (at 8 × 60 K; design 021924) were used instead of the 24 Sure chips. This gives a higher resolution to call chromosomal copy number changes (>10 Mb). Data were analysed for gain or loss of chromosomal copy number changes using CytoGenomics Software v3.0.0.013 according to the manufacturer's instructions (available at www.agilent.com). To ensure hybridization quality controls, female samples hybridized with a male reference DNA (sex mismatch) had to show a consistent gain on chromosome X and a consistent loss of chromosome Y.

Results

The performance of Karyolite BoBs on detecting chromosomal abnormalities in all 22 autosomes and X,Y chromosomes was compared with aCGH in Part I of study. Seventy-seven embryos including 27 cleavage stage embryos and 50 blastocysts were involved in the validation study. A single cell was biopsied from each cleavage stage embryo and 5 to 10 cells were biopsied from each blastocyst. Seventy-five embryos completed both KaryoLite BoBs and aCGH analysis, but two embryos failed the analysis due to one embryo failure in whole genome amplification on Day 3 and one embryo failed labelling on Day 5. The success rate of PGS was 97.4%. The result was 100% concordance compared with aCGH if the resolution is set at chromosome aneuploidy detection (Table 1). One case of partial 9p and 12q deletion identified by aCGH at cleavage stage embryo was missed by KaryoLite BoBs resulting in a false negative rate of 4.3% (Fig. 1). However, as the KaryoLite BoBs is only confined to chromosomal arm resolution, the partial loss of chromosome is beyond the detection limit (down to arm specific resolution). Therefore, we consider there was no false positive case detected. The sensitivity and specificity in detecting aneuploidy is 100%.

Table 2
The spectrum of chromosomal abnormalities detected by KaryoLite BoBs.

	Percentage (n)
Total number of embryo screened by KaryoLite	100% (219)
Total number of euploid embryos	• 28% (62)
Total number of aneuploid embryos	• 72% (157)
Spectrum of aneuploid embryos detected by KaryoLite BoBs	
Single aneuploidy	**34.0% (53)**
Monosomy	7.3% (16)
Trisomy	7.3% (16)
Partial chromosome loss	5.9% (13)
Partial chromosome gain	3.7% (8)
Two aneuploidies	**18% (29)**
Both were complete chromosome loss	0.9% (2)
Both were complete chromosome gain	1.8% (4)
One complete chromosome gain and one complete chromosome loss	1.8% (4)
Both were partial chromosome gain or loss	4.6% (10)
Complete chromosome gain or loss + partial chromosome gain or loss	4.1% (9)
Multiple aneuploidies	**48.0% (75)**
All were complete chromosome loss	0% (0)
All were complete chromosome gain	1.8% (4)
Complete gain and loss in multiple chromosomes	6.8% (15)
All were partial chromosome gain or loss	1.8% (4)
Complete chromosome gain or loss and partial chromosome gain or loss	24.0% (52)

Genetic background

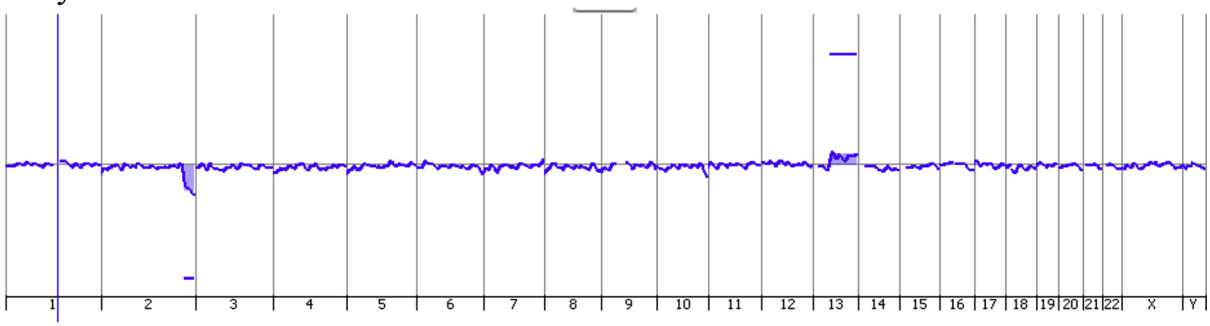
Maternal karyotype: 46,XX,t(2;13)(q33;q12)

Paternal karyotyple: 46XY

Array Type: Agilent G3 Human CGH 8x60K(design 021924)

Reference: Pooled WGA products of a male individual with normal karyotype

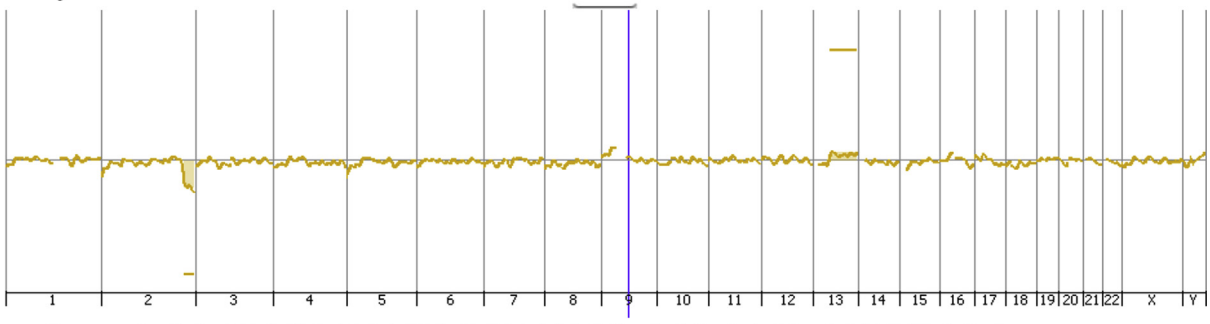
Embryo 1:



Result: arr 2q34q37.3(214683886-243041364)x1,13q14.12q34(45237959-115059020)x3

Aneuploid, XY

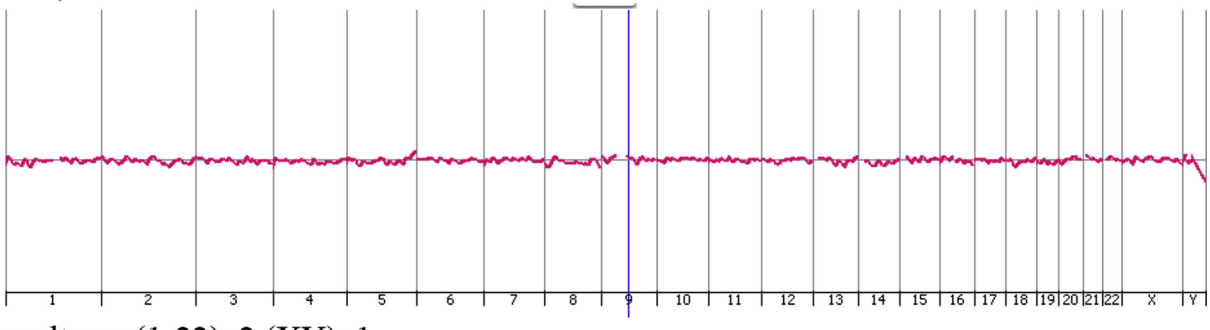
Embryo 2:



Result: arr 2q34q37.3(214354693-243041364)x1,13q14.12q34(45526609-114747979)x3

Aneuploid, XY

Embryo 3:

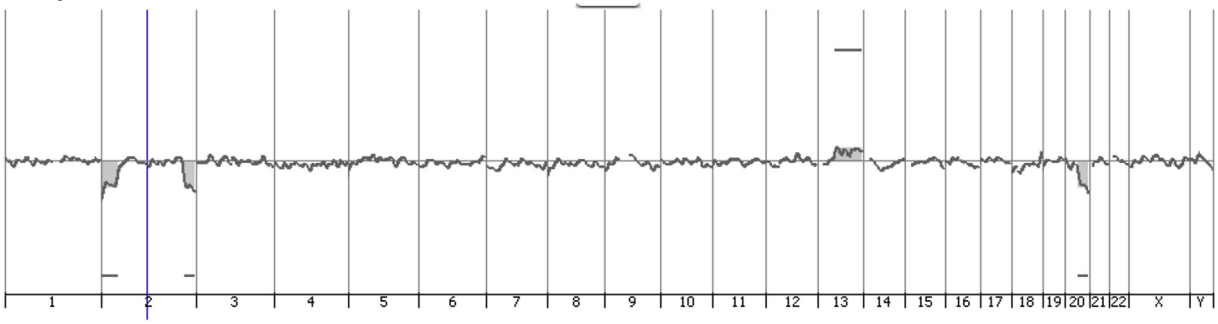


Result: arr (1-22)x2,(XY)x1

Euploid, XY

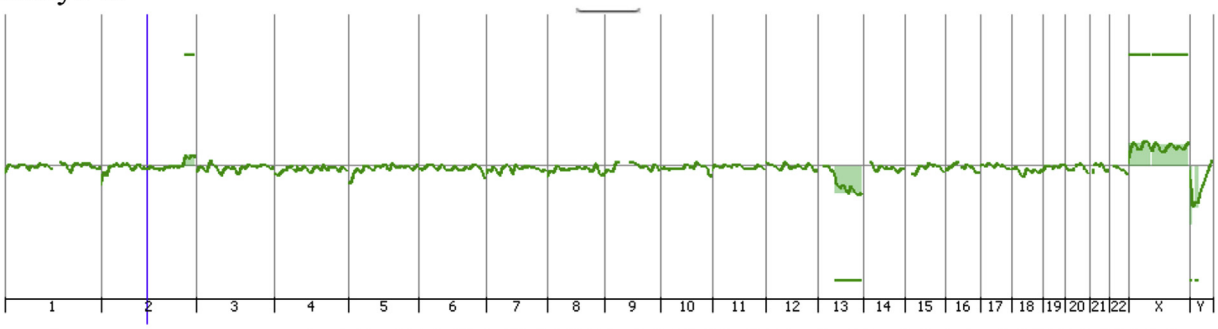
Fig. 2. Illustration of complex chromosomal rearrangement from couple with balanced translocation.

Embryo 4:



Result: arr2p25.3p21(42444-44993698)x1,2q34q37.3(213354074-243041364)x1,13q14.12q34(45554780-113715020)x3, 20q11.23q13(36406083-62872839)x3
Aneuploid, XY

Embryo 5:



Result: arr 2q35q37.3(216212251-242930659)x3,13q14.14q34(45864385-115011507)x1
Aneuploidy, XX

Fig. 2. (*continued*).

The new approach using KaryoLite BoBs as the first line euploid embryo screening tool and followed by arrayCGH for PGS in identifying euploid embryos was evaluated in Part II of the study. A total of 219 embryos undergoing PGS for different indications (including advanced maternal age, recurrent miscarriage, and balanced translocation carriers) were included. The success rate for PGS by KaryoLite BoBs is 100%. KaryoLite BoBs identified 62 euploidies (28%) and 157 aneuploidies. The spectrum of aneuploidy include single, double and multiple aneuploidies (Table 2). KaryoLite BoBs detected euploidy (62/219) were further subject to aCGH analysis. All of these 62 embryos were confirmed to have no gain or loss of whole chromosome copy numbers, but additional structural chromosomal abnormalities were detected in 8% (5/62) by aCGH. And all these five samples were from couples who carried a balanced translocation involving a complex rearrangement less than the size of one p or q arm (Fig. 2). Therefore, a full concordance between KaryoLite BoBs analysis and aCGH was observed for aneuploidies in PGS.

Discussion

While derived from the same cohort of stimulated cycle, sibling embryos can differ in their implantation potential. Conventionally, the developmental potential of embryos has been solely relied on morphological assessment. Although correlations between morphology and implantation rates exist [17], recent studies reported that 30–40% morphologically normal embryos carry chromosomal abnormalities such as aneuploidy [18].

Implantation failure and early miscarriage are usually due to numerical chromosomal abnormalities. (PGS) is increasingly used to select euploid embryo for transfer aiming at higher implantation and pregnancy rates with reduced miscarriage. Recent development of comprehensive chromosome screening (CCS) helps to identify embryos which possess true reproductive potential. Several approaches toward 24-chromosomal analysis including comparative genomic hybridization (CGH)-based microarrays, single nucleotide polymorphism array and quantitative polymerase chain reaction-based techniques have been developed for CCS [19]. A recently published review by Simpson in 2012 suggested that array-CGH (aCGH) was the preferred approach for CCS in PGS [20]. Although aCGH for PGS has been well validated for the detection of aneuploidy or an abnormal number of chromosomes in embryos obtained during IVF treatment [21], this method is expensive and difficult to scale up to meet the increasing demand of IVF cycles.

Our validation study showed that KaryoLite BoBs was robust with low failure rate. This was consistent with results in recent

Table 3
Comparing the cost-effectiveness of arrayCGH versus KaryoLite BoBs based on reagent cost in Hong Kong.

	aCGH	KaryoLite BoBs
Hands-on work (h)	6–10	6
Number of samples per run	4–14	92
Reporting time (day)	2–3	2
Coverage	23 pairs of chromosome down to 10 Mb resolution	23 pairs of chromosome in arm specific
Estimated cost per test*,[a]	~USD 300	~USD 150

* Cost including reagent, equipment maintenance and manpower.
[a] Estimated case calculated based on 10 samples per run.

published papers upon the use of BoBs assay in prenatal cases [10,11,22–25]. Based on the 2823 prenatal cases, the failure rate was less than 4% and was superior to Rapid Aneuploidy Testing for prenatal diagnosis. In our study, we showed that KaryoLite BoBs was equally effective as aCGH in detecting chromosomal abnormalities with a resolution down to chromosome arm level, which was 100% sensitive and specific in detecting aneuploidy. The single case of missing result of 9p and 12q deletion was beyond the detection limit of KaryoLite BoBs. In our retrospective study, only 8% (5/62) of KaryoLite BoBs reported euploid cells had additional structural chromosomal abnormalities by aCGH. In all 5 samples, the embryos were derived from couples carrying balanced translocation with chromosomal rearrangement involving less than one p or q arm i.e. beyond the detection limit of KaryoLite BoB. Also since the couples are balanced translocation carriers they show go for pre-implantation genetic diagnosis rather than PGS. Therefore, KaryoLite BoBs could be a useful cost-effective screening tool to select euploid embryo followed by aCGH to identify small chromosome aberrations to enhance the pregnancy rate from PGS.

Apart from its reliable nature for the detection of aneuploidy, KaryoLite BoBs can match the workflow and cost requirement of worldwide IVF laboratories. The total hands-on time for KaryoLite was estimated to be faster than aCGH. KaryoLite BoBs can process up to 92 samples per analysis, while aCGH can only handle 4–14 samples per analysis [25]. Also the total reagent and consumable cost per sample for KaryoLite was estimated to be less expensive. Thus, the cost-effectiveness of KaryoLite was superior to aCGH in terms of being utilized as a screening tool (Table 3).

The prevalence of parental balanced translocations is relative high in recurrent miscarriage couples (9%) and infertile couples (0.6%) [26]. Couples who carried a balanced translocation are known to have high rates of unbalanced gametes following meiotic segregation and are at risk of producing embryos with unbalanced chromosomes. In fact, embryos with unbalanced translocation have a relatively lower rate of implantation, higher rate of miscarriage, and possibly the birth of offspring with congenital aberrations [27]. Therefore, in selected groups of couple, it is essential to identify the genomically balanced embryo for transfer to maximize their reproductive potential. Traditionally, preimplantation genetic diagnosis (PGD) using fluorescence in situ hybridization (FISH) is the standard for identifying chromosomally balanced embryos from couple with balanced translocation [28]. However, the clinical pregnancy rate was not significantly improved (between 30 and 40%) in IVF with PGD-FISH analysis [29]. This is due to the limitation of PGD-FISH in which it cannot simultaneously test the whole genome for aneuploidy and structural chromosomal imbalance. In fact, as illustrated from our study (Fig. 2), with the effect of meiotic segregation, the chromosomal rearrangements may involve the chromosomes other than the original parental rearranged chromosomes. Since PGD-FISH is specific to known parental translocations, it fails to identify embryos with aneuploidy involving chromosome(s) other than the parental translocations; therefore, it may give a false negative result. The use of BoBs enables a comprehensive 24-chromosome screening for PGS.

Conclusion

KaryoLite BoBs is a robust, accurate method for PGS compared with conventional 24Sure arrayCGH, with a resolution down to chromosome arm level. KaryoLite BoBs could be widely applied in PGS to select euploid embryos for transfer or act as the first tier PGS screening step before aCGH. Our data is the first study to validate this high throughput, cost-effective approach that can be applied to both Day-3 and Day-5 aneuploidy analysis to improve implantation rate after PGS. Besides, the use of aCGH after BoB in our protocol enables the identification of complex chromosomal rearrangement from couples with balanced translocation.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

[1] de Mouzon J, Goossens V, Bhattacharya S, Castilla JA, Ferraretti AP, Korsak V, et al. Assisted reproductive technology in Europe, 2006: results generated from European registers by ESHRE. Hum Reprod 2010;25(8):1851–62.
[2] Nygren KG, Sullivan E, Zegers-Hochschild F, Mansour R, Ishihara O, Adamson GD, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) world report: assisted reproductive technology 2003. Fertil Steril 2011;95(7):2209–22. 2222 e1–17.
[3] CDC. 2008 assisted reproductive technology success rates: national summary and fertility clinic reports. Centers for Disease Control and Prevention; 2011. Available from: https://www.cdc.gov/art/pdf/archived/art_2008_full.pdf.
[4] Bielanska M, Tan SL, Ao A. Chromosomal mosaicism throughout human preimplantation development in vitro: incidence, type, and relevance to embryo outcome. Hum Reprod 2002;17(2):413–9.
[5] Magli MC, Gianaroli L, Ferraretti AP, Lappi M, Ruberti A, Farfalli V. Embryo morphology and development are dependent on the chromosomal complement. Fertil Steril 2007;87(3):534–41.
[6] Munne S, Alikani M, Tomkin G, Grifo J, Cohen J. Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. Fertil Steril 1995;64(2):382–91.
[7] Munne S, Chen S, Colls P, Carrisi J, Zheng X, Cekleniak N, et al. Maternal age, morphology, development and chromosome abnormalities in over 6000 cleavage-stage embryos. Reprod Biomed Online 2007;14(5):628–34.
[8] Munne S, Lee A, Rosenwaks Z, Grifo J, Cohen J. Diagnosis of major chromosome aneuploidies in human preimplantation embryos. Hum Reprod 1993;8(12):2185–91.
[9] Dahdouh EM, Balayla J, Garcia-Velasco JA. Impact of blastocyst biopsy and comprehensive chromosome screening technology on preimplantation genetic screening: a systematic review of randomized controlled trials. Reprod Biomed Online 2015;30(3):281–9.
[10] Vialard F, Simoni G, Aboura A, De Toffol S, Molina Gomes D, Marcato L, et al. Prenatal BACs-on-Beads: a new technology for rapid detection of aneuploidies and microdeletions in prenatal diagnosis. Prenat Diagn 2011;31(5):500–8.
[11] Gross SJ, Bajaj K, Garry D, Klugman S, Karpel BM, Roe AM, et al. Rapid and novel prenatal molecular assay for detecting aneuploidies and microdeletion syndromes. Prenat Diagn 2011;31(3):259–66.
[12] Grati FR, Gomes DM, Ganesamoorthy D, Marcato L, De Toffol S, Blondeel E, et al. Application of a new molecular technique for the genetic evaluation of products of conception. Prenat Diagn 2013;33(1):32–41.
[13] Baxter L, Adayapalam N. A comparative study of standard cytogenetic evaluation and molecular karyotyping for products of conception. Diagn Mol Pathol 2013;22(4):228–35.

[14] Paxton CN, Brothman AR, Geiersbach KB. Rapid aneusomy detection in products of conception using the KaryoLite BACs-on-Beads assay. Prenat Diagn 2013;33(1):25–31.
[15] Sheath KL, Duffy L, Asquith P, Love DR, George A M. Bacterial artificial chromosomes (BACs)-on-Beads as a diagnostic platform for the rapid aneuploidy screening of products of conception. Mol Med Rep 2013;8(2):650–4.
[16] Fiorentino F, Caiazzo F, Napolitano S, Spizzichino L, Bono S, Sessa M, et al. Introducing array comparative genomic hybridization into routine prenatal diagnosis practice: a prospective study on over 1000 consecutive clinical cases. Prenat Diagn 2011;31(13):1270–82.
[17] Borini A, Laqalla C, Cattoli M, Sereni E, Sciajino R, Flamigni C, et al. Predictive factors for embryo implantation potential. Reprod Biomed Online 2005;10(5):653–68.
[18] Baltaci V, Satiroglu H, Kabukcu C, Unsal E, Aydinuraz B, Uner O, et al. Relationship between embryo quality and aneuploidies. Reprod Biomed Online 2006;12(1):77–82.
[19] Handyside AH. 24-chromosome copy number analysis: a comparison of available technologies. Fertil Steril 2013;100(3):595–602.
[20] Simpson JL. Preimplantation genetic diagnosis to improve pregnancy outcomes in subfertility. Best Pract Res Clin Obstet Gynaecol 2012;26(6):805–15.
[21] Rubio C, Rodrigo L, Mir P, Mateu E, Peinado V, Milan M, et al. Use of array comparative genomic hybridization (array-CGH) for embryo assessment: clinical results. Fertil Steril 2013;99(4):1044–8.
[22] Shaffer LG, Coppinger J, Morton SA, Alliman S, Burleson J, Traylor R, et al. The development of a rapid assay for prenatal testing of common aneuploidies and microdeletion syndromes. Prenat Diagn 2011;31(8):778–87.
[23] Vialard F, Simoni G, Gomes DM, Abourra A, De Toffol S, Bru F, MArtinez Romero MC, et al. Prenatal BACs-on-Beads: the prospective experience of five prenatal diagnosis laboratories. Prenat Diagn 2012;32(4):329–35.
[24] Cheng YK, Wong C, Wong HK, Leung KO, Kwok YK, Suen A, et al. The detection of mosaicism by prenatal BoBs. Prenat Diagn 2013;33(1):42–9.
[25] Choy RK, Chen Y, Sun XF, Kwok YK, Leung TY. BACs-on-beads: a new robust and rapid detection method for prenatal diagnosis. Expert Rev Mol Diagn 2014;14(3):273–80.
[26] Stern C, Pertile M, Norris H, Hale L, Baker HW. Chromosome translocations in couples with in-vitro fertilization implantation failure. Hum Reprod 1999;14(8):2097–101.
[27] Escudero T, Estop A, Fischer J, Munne S. Preimplantation genetic diagnosis for complex chromosome rearrangements. Am J Med Genet A 2008;146A(13):1662–9.
[28] Munne S, Sandalinas M, Escudero T, Fung J, Gianaroli L, Cohen J. Outcome of preimplantation genetic diagnosis of translocations. Fertil Steril 2000;73(6):1209–18.
[29] Harper JC, Wilton L, Traeger-Synodinos J, Goossens V, Moutou C, SenGupta SB, et al. The ESHRE PGD Consortium: 10 years of data collection. Hum Reprod Update 2012;18(3):234–47.